Parallel transport of biological cells using individually addressable VCSEL arrays as optical tweezers

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Abstract

We have demonstrated the use of vertical cavity surface emitting lasers (VCSELs) for optical trapping and active manipulation of live biological cells and microspheres. We have experimentally verified that the Laguerre–Gaussian laser mode output from the VCSEL functions just as well as the traditional Gaussian fundamental laser mode for optically trapping biological cells and may be preferable since the highest intensity of the Laguerre–Gaussian mode is located at the outer ring of the optical aperture, which allows for stronger optical confinement to be obtained for a lower total power. Another advantage that VCSELs have over conventional gas and diode lasers is their ability to be manufactured in an array form. Using a 2×2 array of VCSELs, the simultaneous and independent transport of four human red blood cells is demonstrated indicating that much larger two-dimensional VCSEL arrays can be used as individually addressable optical tweezers in biological chips and systems. This parallel transport capability will have a significant impact in currently developing biochip array and assay technologies through the facilitation of the selection, relocation, and precision placement of cells.
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1. Introduction

The technique of all-optical micromanipulation of small objects and biological samples through the use of optical tweezers has become a common and useful scientific tool [1–4]. Experiments based on the use of optical tweezers have demonstrated precise manipulation of living cells and organelles within cells [5], as well as discovery of the function and forces imparted by molecular motors on such biological molecules as RNA and DNA [6,7]. In the rapidly developing field of microfluidic lab-on-a-chip devices where the capability of transporting large arrays of biological samples in parallel can aid, for example, the processing of cellular assays, it is essential that the controlling devices be small and have the ability of being replicated in large arrays to accommodate the biological need of performing multiple, simultaneous experiments in parallel.

Recently, we have reported on a technique whereby a standard packaged Hermite–Gaussian spatial mode VCSEL may be converted to a Laguerre–Gaussian mode laser through a simple post-fabrication current annealing process [8]. At higher orders, Laguerre–Gaussian modes will tend to resemble a toroidal shape with most of the optical power located in the outer ring of the mode. It has been demonstrated both theoretically and experimentally that Laguerre–Gaussian mode lasers can offer a significant advantage over fundamental Gaussian mode lasers for three-dimensional optical trapping [9–11]. In a tightly focused beam, the greatest axial restoring force to the three-dimensional trap comes from the photons at the largest incidence angle. Photons in the center portion of the beam have the deleterious effect of producing an axial scattering force along the direction of propagation that drives the object out of the trap. By removing power from the central portion of the beam the optical trap strength will improve along the beam axis.

In this article, we report and analyze the novel use of vertical cavity surface emitting laser (VCSEL) arrays for the independently controlled, parallel capture and transport of large numbers of individual biological cells. For a single VCSEL beam optical tweezer, we have found that the types of cells that can be manipulated will vary according to the ratio of the maximum output power of the VCSEL to the cell's radius and its relative index of refraction with respect

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to the surrounding media. The use of VCSEL arrays provide the advantages of being compact, individually addressable, potentially very inexpensive, and compatible with other optoelectronic functions that may be desirable for biological lab-on-a-chip devices. In addition, VCSELs can be designed to operate at different wavelengths of interest ranging from the blue, using wide bandgap materials [12], to the infrared. Furthermore, it has been demonstrated that VCSELs can be made tunable [13] and therefore, can find use in various biophotonics functions such as fluorescence excitation or spectroscopic analysis.

2. Experimental

The near infrared wavelength regime at 850 nm is desirable for optical tweezers because it resides in a low absorption range for biological specimens and the surrounding buffer solution [14]. In our experiments, we have characterized the transverse optical trapping force of 850 nm wavelength, 15 μm aperture, proton-implant VCSEL devices (Honeywell Inc.) and demonstrated the independent transport of up to four human red blood cells with a 2 × 2 array of these VCSELs. The experimental setup is shown in Fig. 1. The optical tweezer trap is constructed using a side-mounted microscope-type design where the output from the VCSEL array is focused into the sample through a 100×, 1.25 NA oil-immersion microscope objective. The use of a side-mounted microscope design aids in the trapping of cells and particles by increasing the time before the particles settle and adhere to the bottom surface of the sample due to gravity, i.e. the cells flow in a direction parallel to the glass surface versus the perpendicular direction of a conventional tweezer setup. The sample, which is deposited on a
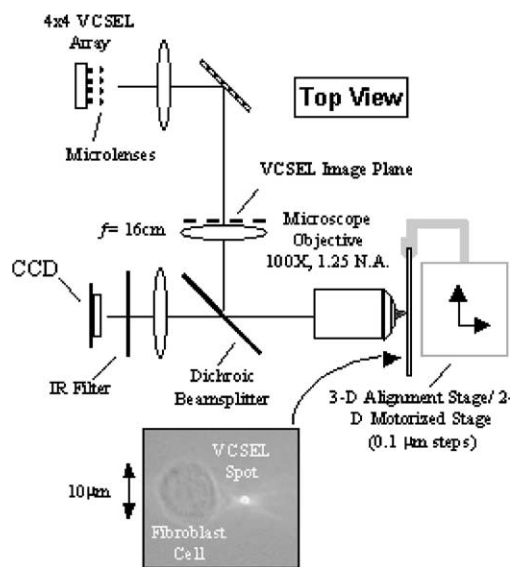

Fig. 1. Top view of the experimental setup of optical tweezers in a side-mounted microscope design. Inset shows an image of a single VCSEL tweezer spot next to a 10 μm diameter murine 3T3 fibroblast cell.

microscope cover slip, is mounted on a three-dimensional alignment stage, which subsequently resides on a two-dimensional linear stepper motor stage (0.1 μm step size) to allow variable-speed translation by which the transverse forces can be measured. This setup is not suitable for making accurate measurements of trap strength along the axial beam direction due to the increase in spherical aberration that is observed at increasing depths within a sample, since the high numerical aperture microscope objective in our optical system is aberration compensated for a specific working distance. The spherical aberration that inhibits axial tweezer force measurements at increasing depths can be alleviated by using a microscope objective with an adjustable ring compensator (Nikon Inc.). For our experiments though, it was acceptable to limit ourselves to the current optical system since, we concentrated on performing parallel pick-and-place operations in which the depth is usually fixed and lateral positioning of objects or cells is desired. We did, however, verify in all cases that a fully three-dimensional trap was achieved by translating the object along all three axes (axial and lateral) and by observing that Brownian motion alone was insufficient to permit the cells to escape while the trap was stationary.

Measurements of the transverse trapping force of the VCSEL tweezer was performed using a 10,000–1 dilution of stock yeast cells (Fleischmann's) and human red blood cells (San Diego Blood Bank) immersed in Alsever's solution, and a sparse concentration of 5 and 10 μm polystyrene microspheres (Bangs Laboratories Inc.) immersed in DI water. To measure the optical tweezer force, a trapped particle is translated in a transverse direction through the fluid at increasingly higher speeds until the fluidic drag force exceeded the optical trapping force. Assuming spherical object symmetry and laminar fluid flow, the fluidic drag on an object is determined from the Navier–Stokes equation and at the critical velocity, v_c, the optical trapping force is equal to the fluidic drag force such that

$$F_{opt} = F_{drag} = 6\pi\eta r v_c$$

where the viscosity η for water is ($10E{-}3$ N s/m^3) and r is the particle radius [15,16].

3. Results

The maximal transverse drag force, and hence the maximal optical trapping force, achieved by the VCSEL tweezer in our system is 5.7 pN for the 10 μm diameter beads, 1.8 pN for the 5 μm diameter beads, 0.79 pN for the 5μm diameter human red blood cells, and 2.7 pN for the 4 μm yeast cell, at the maximum achievable tweezer power of 3.0 mW in the sample. Fig. 2 shows a graph of the critical transverse velocities at which the objects fall out of the trap (escape velocity) as a function of VCSEL tweezer power. In addition, the variations in optical trapping force, calculated by solving the Navier–Stokes equations using the escape velocity measurements, are
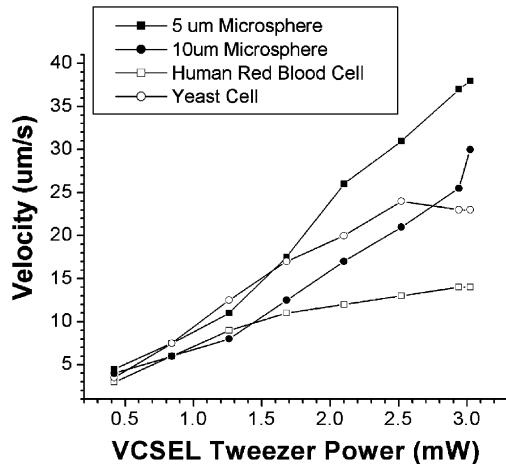
Fig. 2. Plot of the maximum achievable transverse velocities for the objects at a set VCSEL tweezer power level. It can be seen that the object's transverse velocity will vary according to both the size and index of refraction of the object. For the three objects that have approximately the same size, the velocity will increase as a function of the object's relative index of refraction, starting with the human red blood cell that has the smallest relative index of refraction ($n = 1.36$), continuing with the yeast cell ($1.36 \leq n \leq 1.40$), and peaking with the 5 μm microsphere ($n = 1.59$). It can be further noted that in the case of the 5 and 10 μm microspheres which have the same relative index of refraction, the maximum velocities will decrease as a function of increasing size due to the radial dependence of the fluid drag force.

graphed in Fig. 3 as a function of VCSEL tweezer power for the cells and the polystyrene microspheres. As can be seen in the graphs, the smaller diameter beads may be dragged at a higher velocity than the larger diameter beads although the actual strength of the optical trap is higher for the larger
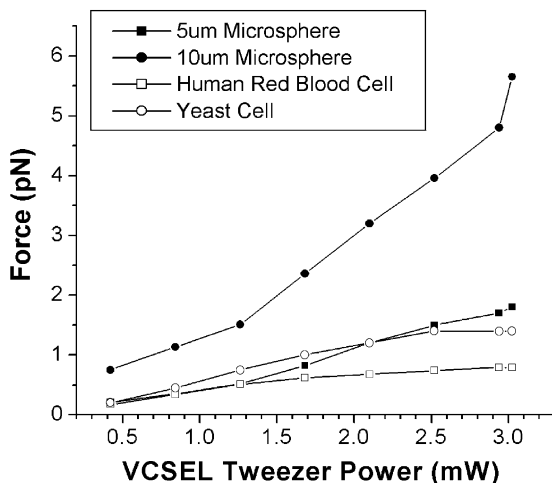

Fig. 3. Plot of the transverse optical trapping force as a function of the VCSEL optical tweezer power in the sample for 5 and 10 μm polystyrene microspheres, human red blood cells, and yeast cells. As seen from the graph, the objects with a larger relative index of refraction ($n = 1.59$ for polystyrene, $n = 1.36$ for the human RBC, and $n = 1.36$–1.40 for the yeast cell) exhibit a higher optical trapping force. Additionally, the difference in trapping force between the 5 and 10 μm microspheres signifies that the optical trapping force increases with the size of the object.

diameter spheres. This can be explained by noting that the fluid drag force will increase more rapidly than the VCSEL tweezer force (at constant power) as a function of the microsphere radius. One other point to take into consideration is that the trapping force that can be achieved on a biological cell is much smaller than that of the polystyrene beads due to the lower average refractive index of the cells (∼1.36–1.40 for the cells versus 1.59 for the microspheres) and the fact that the cell's shape can deviate from a that of perfect sphere. As the sizes of the cells increases, it becomes more difficult to trap the biological cells due to their low relative indices of refraction and the limitation of maximum output power (∼5–7 mW) that can be achieved by the VCSELs used in this experiment. By combining the output of several VCSELs into a single trap, it is possible that the optical tweezer power can be further increased, thus increasing the optical trapping force. In addition, a larger area VCSEL, although multimode, has the ability to provide more power than a single-mode VCSEL, which in principle makes it advantageous when manipulating larger objects.

Using human red blood cells and yeast cells, experiments were conducted contrasting the transverse optical trapping force of a fundamental Gaussian mode tweezer, generated by a GaAlAs diode laser (Melles Griot), with the force of a VCSEL Laguerre–Gaussian mode tweezer. Both the diode laser and VCSEL have an operating wavelength of 850 nm and Fig. 4 shows the comparison of the optical trapping force of the fundamental Gaussian mode tweezer and the Laguerre–Gaussian mode tweezer as a function of the optical tweezer
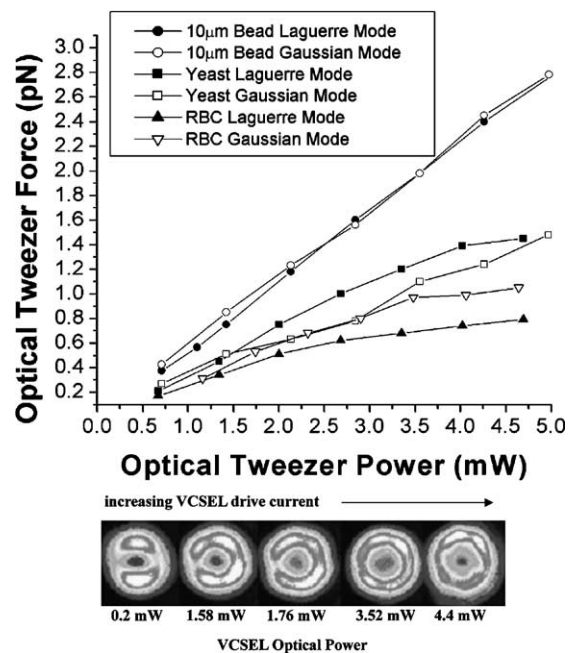

Fig. 4. Comparison of the transverse optical trapping force obtained from a VCSEL Laguerre–Gaussian mode tweezer versus a fundamental Gaussian mode tweezer originating from a diode laser for a human red blood cell, a yeast cell, and a 10 μm polystyrene microsphere. The picture shown below the graph reveals how intensity distribution in the output mode structure of the VCSEL changes as a function of the drive current.

power. Examining Fig. 4, we see that for the two cells and the 10 μm polystyrene microsphere, the transverse optical trapping force of the VCSEL Laguerre–Gaussian mode tweezer is comparable to that of the fundamental Gaussian mode tweezer. From these results, we can conclude that for biological cells, the VCSEL optical tweezer performs equally as well as the diode laser optical tweezer with respect to the transverse optical trapping forces. Additionally, Fig. 4 shows the changing mode shape of the VCSEL Laguerre–Gaussian mode as a function of the driving current. As the driving current increases, the intensity of the VCSEL Laguerre–Gaussian mode becomes more concentrated at the outer ring of the mode resulting in an increase in the axial optical trapping force that has been shown to create greater optical trap stability when compared to the fundamental Gaussian mode [11].
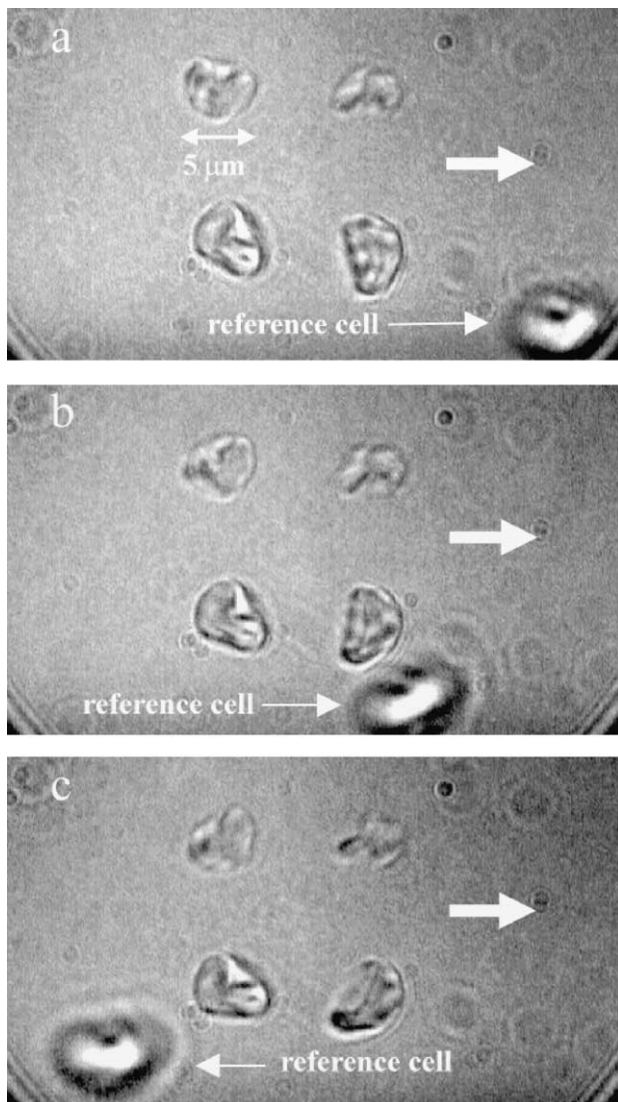
The parallel array transport capability of the VCSEL devices is demonstrated with a 2 × 2 grouping of VCSELs from a 4 × 4 Honeywell VCSEL array. The purpose of only using four out of a possible 16 VCSELs in the array can be attributed to the 250 μm device pitch of the array. At this fixed pitch, it is difficult to simultaneously maintain any appreciable spacing of the optical tweezers in the sample plane and fill the back-aperture of the microscope objective; which is necessary in order to create the tightly focused beam required for optical trapping. By choosing the corner VCSELs in the array, we were able to increase the pitch of the VCSELs to 750 μm, which is sufficient to fill the back-aperture of the microscope objective. The beams from the VCSEL array are then collected and imaged together through the microscope objective resulting in an inter-trap spacing of 15 μm. Fig. 5 shows sequential images captured by the CCD camera of four optically trapped human red blood cells undergoing simultaneous translation. As can be seen from the images, the relative spacing between the four cells remains constant as the cells are moved to the right relative to the reference cell at a speed of 10 μm/s. Additionally, Fig. 6 demonstrates the independent control of each optical tweezer through the release of cells

Fig. 5. Sequence of images showing the trapping and simultaneous translation of four human red blood cells using a 2 × 2 VCSEL array. The cells are moving to the right relative to the position of the reference cell at a speed of 10 μm/s.
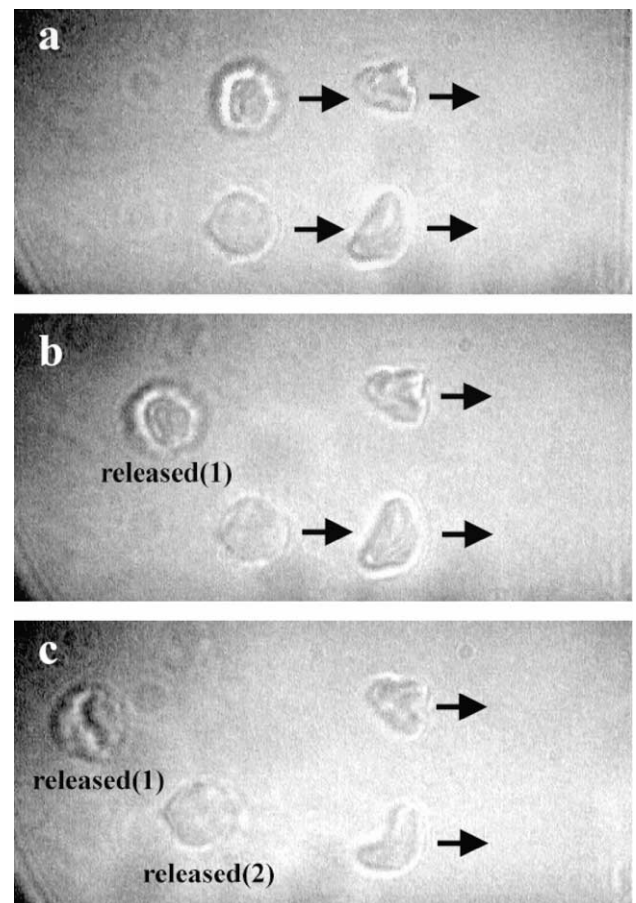

Fig. 6. Sequence of images through which independent control of each optical tweezer in the array is demonstrated by performing a parallel pick-and-place operation, which involves turning off the VCSEL and releasing the corresponding cell from its trap at a particular location.

from the trap at specific intervals by turning off the corresponding VCSEL in the array.

4. Conclusion

As a result of our experiments, we demonstrate that for biological cells, the higher order Laguerre–Gaussian mode VCSEL tweezers have comparable transverse optical trapping forces when contrasted with the fundamental mode Gaussian laser tweezer and greater axial optical trap strength through a mode structure that minimizes the photon scattering force inside the optical trap. With this knowledge, we then incorporated an array of VCSELs into an optical tweezer system in order to show that the 850 nm VCSELs are able to optically capture and manipulate biological cells and polystyrene microspheres in a parallel and individually controllable fashion. The high device density and compactness achievable with VCSEL arrays makes them favorably suited for optical micromanipulation applications where individual manipulation of cells and high throughput are necessary, such as multi-object parallel transport in biochip array technologies.

Acknowledgements

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